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Myeloid translocation gene 16b is a dual A-kinase anchoring protein that interacts selectively with plexins in a phospho-regulated manner

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ABSTRACT

The myeloid translocation gene (MTG) homologue Nervy associates with PlexinA on the plasma membrane, where it functions as an A-kinase anchoring protein (AKAP) to modulate plexin-mediated semaphorin signaling in *Drosophila*. Mammalian MTG16b is an AKAP found in immune cells where plexin-mediated semaphorin signaling regulates immune responses. This study provides the first evidence that MTG16b is a dual AKAP capable of binding plexins. These interactions are selective (PlexinA1 and A3 bind MTG, while PlexinB1 does not) and can be regulated by PKA-phosphorylation. Collectively, these data suggest a possible mechanism for the targeting and integration of adenosine 3′,5′-cyclic monophosphate (cAMP) and semaphorin signaling in immune cells.

Structured summary:
MINT-7556975: *PlexinA3* (uniprotkb:P51805) *physically interacts* (MI:0915) *with MTG 16b* (uniprotkb:O75081) *by anti tag coimmunoprecipitation* (MI:0007)
MINT-7557008: *RI alpha* (uniprotkb:Q9DBC7) *physically interacts* (MI:0915) *with MTG 16b* (uniprotkb:O75081) *by anti bait coimmunoprecipitation* (MI:0006)
MINT-7556989: *MTG 16b* (uniprotkb:O75081) *physically interacts* (MI:0915) *with PlexinA3* (uniprotkb:P51805) *by pull down* (MI:0096)

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1. Introduction

Agents that activate the adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent second messenger pathway are potent inhibitors of T-cell activation [1,2]. While numerous reports document the effectiveness of cAMP as an anti-inflammatory agent, the molecular mechanisms producing these effects are still under investigation. This lack of knowledge has thwarted progress toward clinical application of therapies that target cAMP. A-kinase anchoring proteins (AKAPs) are defined by their ability to bind one or more of the regulatory subunits (type I: RIα and RIβ and type II: RIIα and RIIβ) of cAMP-dependent protein kinase A (PKA). These subunits interact with an amphipathic helix domain on the AKAP. AKAPs target the action of PKA signaling by acting as scaffolding proteins, spatially restricting function by simultaneously binding related signal transduction enzymes [3,4]. We have identified seven different AKAPs in T lymphocytes and dendritic cells, including the discovery of a novel AKAP, myeloid translocation gene (MTG) [4,5]. MTG was originally identified as a fusion protein with AML in patients with acute myeloid leukemia and has been detected in the nucleus, cytoplasm and Golgi [6]. MTG acts as an regulatory subunit of type II PKA (RII) binding AKAP and is thus a potential adaptor protein for cAMP signaling in immune response [4,7]. However, a growing body of evidence suggests that the type I PKA isoforms may play a greater role in regulating the immune response; mice lacking RIIα have normal immune responses to cAMP, type I regulatory subunits co-localize with the TCR during T-cell activation [8], and activation of PKA type I alpha alone is sufficient for cAMP-dependent immunosuppression [9]. Thus, it would be interesting to determine whether MTG is a dual AKAP.

Controlling the concentration of cAMP and the activity of PKA is crucial for directing an axon to its proper target [10]. Insight into how cAMP dictates axonal steering responses has been gained from the discovery that Nervy, a *Drosophila* AKAP with significant homology to MTG, couples plexin to PKA to modulate semaphorin

Abbreviations: AKAP, A-kinase anchoring protein; cAMP, adenosine 3′,5′-cyclic monophosphate; GFP, green fluorescent protein; GST, glutathione S-transferase; IB, immunoblot; IP, immunoprecipitation; MTG, myeloid translocation gene; PKA, cAMP-dependent protein kinase; RI, regulatory subunit of type I PKA; RII, regulatory subunit of type II PKA

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repulsion. Work by Terman and Kolodkin illustrates that Nervy associates with PlexinA on the plasma membrane. Nervy functions as an AKAP and modulates intracellular signaling initiated by the interaction of semaphorins and plexins [11]. This finding suggests a mechanism for the integration of diverse signaling inputs to the axonal growth cone [11,12].

The immune and nervous systems are similar in many respects. Both are highly networked systems that interact using shared molecules such as chemical mediators and cytokines [13]. T-cells and antigen presenting cells (APCs) form a unique cellular architecture at their contact zone (the immunological synapse) that is structurally similar to the neurological synapse [14]. Several semaphorins have been detected in cells of the immune system and have been shown to be key regulatory molecules controlling the immune response, reviewed in [13,15–17]. The plexin and neurophilin families of semaphorin receptor proteins are also expressed by a variety of immune cells and are involved in semaphorin signaling in the immune system [16,18–22].

In this study, we investigate whether mammalian proteins MTG16b and plexins can interact. Results indicate that MTG binds selectively to plexins, and that this binding can be regulated by PKA-phosphorylation. Additionally, we determine that MTG is a dual AKAP, capable of binding both regulatory subunit of type I PKA (RI) and RII. Taken together, these data indicate that MTG16b has the potential to scaffold cAMP and semaphorin signaling in immune cells.

2. Materials and methods

2.1. Vector constructs

Preparation of MTG16b constructs is described previously [4,23]. Constructs expressing full-length human PlexinA3-pcDNA, PlexinB1-pcDNA, and human/mouse chimera PlexinA1-pcDNA (all VSV-G tagged) were kindly provided by Dr. Luce Tamagnone. For bacterial expression, restriction enzyme digestion was used to obtain just the cytoplasmic domain of each plexin: XhoI for PlexinA1, EcoRI and NcoI for PlexinA3 and EcoRI for PlexinB1. Fragments were ligated into pET30 (S-protein-tagged) or pGEX-5X [glutathione S-transferase (GST)-tagged]. For PlexinA3 cloning into pGEX-5X, primers were designed to add BamHI and NotI restriction sites, respectively: forward 5′-CGCGGATCCATGCCATGGTGG CCCTGCAGAGC-3′ and reverse 5′-ATAAGAATGCGGCCGCCCTCCTCA CCGATTCCACCAC-3′. Due to poor protein expression using PlexinB1-pcDNA to transfect COS7 cells, PlexinB1 was also expressed in pEGFP (cut from pcDNA construct as above).

2.2. Transformation, expression and pulldown assays

Methods for transformation, bacterial protein induction and expression, and pulldown assays are described previously [24].

2.3. Transfection and immunoprecipitation (IP) and immunoblot (IB)

Transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) as described previously [24], using 2.5 μg of MTG-pcDNA (myc-his tagged) and 5 μg of plexin-pcDNA (VSV-G tagged). Immunoprecipitation and immunoblot methods are described in [24], with modifications. Briefly, a glycerol lysis buffer was used (20 mM Tris–HCl pH 7, 4, 5 mM EDTA, 150 mM NaCl, 10% glycerol, 1% Triton-X 100, and protease inhibitors as in [24]. IP antibodies were: rabbit polyclonal anti-VSV-G (8 μg; Sigma–Aldrich, St. Louis, MO), mouse monoclonal anti-myc (10 μg; Santa Cruz Biotechnology, Santa Cruz, CA; used to IP MTG rather than PlexinB1-GFP in co-IP experiments due to the myc antibody's superior performance in IP assays compared to the green fluorescent protein (GFP) antibody), or mouse monoclonal anti-RI (4 μg, BD Biosciences, San Jose, CA). Isotype appropriate IgG (all BD Biosciences) in amounts equal to IP antibody was used as a negative control in each IP. IB antibodies were VSV-G (1:2000), myc (1:500), RI (1:300), and rabbit polyclonal anti-GFP (1:250; Clontech, Mountain View, CA). Genscript (Piscataway, NJ) One Hour IP Western Kit was used according to manufacturer's instructions for RI western blots to minimize interference from IP/IgG cross reaction with the IB antibody.

2.4. Phosphorylation

Phosphorylation for pulldown experiments is described in [3].

3. Results/discussion

Unless otherwise specified, proteins in this section are recombinant and tagged to facilitate IPs and IBs. Appropriate controls are included to ensure that binding is specific to the protein and not the tags. See figure legends for detail.

3.1. MTG interacts with plexins

To determine whether MTG interacts with plexins, two types of binding assays were performed. Co-immunoprecipitation experiments using co-transfected COS7 cells revealed that full-length (FL) MTG interacts with plexins A1 and A3, but not PlexinB1 (Fig. 1A, compare lane 3 in upper panels). To confirm the results of IP experiments using a second method, to delineate the binding domains involved, and to examine the effects of phosphorylation on binding, MTG16b (FL and fragments: amino acids 200–700, 700–1510, and 1510–2000) was bacterially expressed. Due to the fact that MTG is not a membrane or extracellularly expressed protein, we hypothesized that it would interact with the highly conserved cytoplasmic domain of plexins [4]. As such, we subcloned and expressed just the cytoplasmic domains (C1 and C2) of plexins A1, A3 and B1. Consistent with co-immunoprecipitation assays, the cytoplasmic domains plexins A1 and A3 bound to FL MTG16b, while PlexinB1 did not (Fig. 1B, compare lane 3 in upper panels). Interestingly, in fragment pulldowns, plexins A1 and A3 bound well to FL MTG, but they both bound only weakly and inconsistently to the MTG fragments (primarily binding to the 200–700 and 700–1510 fragments, data not shown). This may indicate that proper folding of the intact FL MTG protein is required for optimal binding of plexins.

3.2. MTG is a dual AKAP

We used co-IP and pulldown assay to determine whether MTG interacts with RI. Co-IP experiments using MTG transfected COS7 cells revealed that MTG interacts with endogenously expressed RI (Fig. 2A). To confirm a direct interaction, purified RIα and MTG were used in pulldown assays. Consistent with co-immunoprecipitation assays, RIα interacts with FL MTG (Fig. 2B). Together with our previous studies that demonstrate RII-MTG interactions [4], these data indicate that MTG is a dual AKAP. This result is supported by recently published sequence analyses suggesting that in addition to the well-characterized RII-binding amphipathic helix domain, dual AKAPs contain a PKA binding region called the RI Specifier Region (RISR) [25]. Sequence alignment with the dual AKAPs presented in the Jarnaess study indicate that MTG16b contains the RISR motif (Supplementary data).

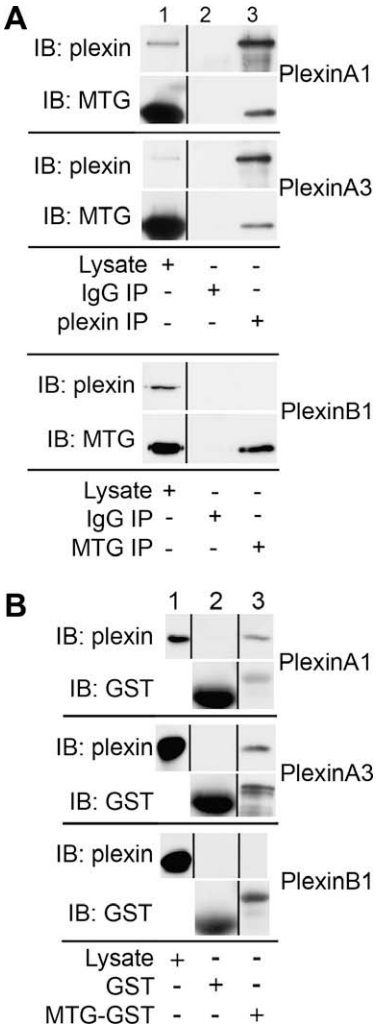

Fig. 1. MTG interacts with PlexinA1 and PlexinA3, but not PlexinB1. (A) COS7 cells were co-transfected with full-length MTG-pcDNA(myc-His) and PlexinA1-pcDNA, PlexinA3-pcDNA (both VSV-G tagged), or PlexinB1-pEGFP. Lane 1 in all panels is a lysate control for transfection. In the PlexinA1 and PlexinA3 panels, immunoprecipitations (IP) were performed with negative control IgG (lane 2) or VSV-G polyclonal antibody (lane 3). After SDS-PAGE and transfer, membranes were cut in half and immunoblotted (IB) with anti-VSV-G antibody to confirm IP (top panel), or anti-myc antibody (bottom panel), demonstrating that MTG co-IP with plexins A1 and A3. In the PlexinB1 panel, IPs were performed with negative control IgG (lane 2) or anti-myc monoclonal antibody (lane 3). Anti-myc antibody IB was performed to confirm IP, and polyclonal anti-GFP antibody IB was performed, demonstrating that MTG does not co-IP with PlexinB1. (B) Pulldown assays using bacterially expressed proteins and glutathione sepharose (GST) beads. Lane 1 in each panel is the plexin-S tagged protein. Plexin-S tagged protein lysates were incubated with GST beads alone (data not shown), GST beads bound to GST lysates (lane 2), or MTG-GST lysates (lane 3). Western analysis was used to detect binding of plexins (upper blot in each panel) to GST (lower blot in each panel). In (A) and (B) the results are representative blots of three independent experiments.

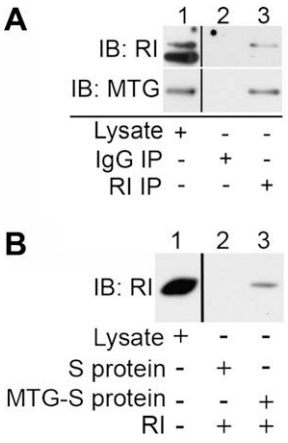

Fig. 2. MTG interacts with RI. (A) COS7 cells were transfected with full-length MTG-pcDNA(myc-His). Lane 1 in both panels is a lysate control to demonstrate size and confirm transfection. IPs were performed with negative control IgG (lane 2) or RI monoclonal antibody (lane 3). After SDS-PAGE and transfer, membranes were cut in half and immunoblotted (IB) with anti-RI antibody to confirm IP (top panel), or anti-myc antibody (bottom panel) to demonstrate that MTG co-IPs with RI. Both IBs used Genscript's (Piscataway, NJ) one-step IP Western blot kit. Since different antibodies are used, resulting bands should not be considered quantitative measures of protein. (B) Pulldown assays using S-protein beads. Lane 1 is RIα protein bacterial lysate compared to pulldown lanes 2 and 3. Bacterially expressed, affinity-purified RIα was incubated with S-protein beads alone (data not shown), S-protein beads bound to S-protein bacterial lysates (lane 2), or MTG-S protein bacterial lysates (lane 3). Western analyses with anti-RIα monoclonal antibody were used to detect RI binding. In (A) and (B) the results are representative blots of three independent experiments.

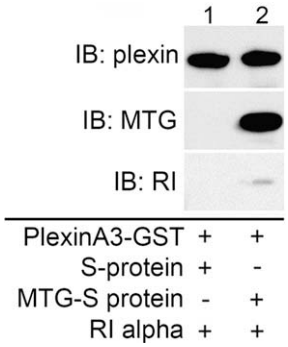

Fig. 3. MTG can form a complex with plexin and RIα. In modified pulldown assays, PlexinA3-GST was immobilized on GST beads and incubated with either negative control S-protein (lane 1) or MTG-S protein (lane 2). Both sets of beads were then washed and incubated with bacterially expressed, cAMP affinity-purified RIα (lanes 1 and 2). The samples were separated by SDS–PAGE, transferred to Immobilon PVDF membranes, and western blots were performed with anti-RIα monoclonal antibody and goat anti-mouse-HRP-conjugated secondary antibody in order to detect binding of RI (lower blot). Anti-GST-HRP and anti-S protein-HRP western blots were also performed in order to confirm loading of PlexinA3 (upper blot) and MTG (middle blot), respectively. These results are representative blots of three independent experiments.

3.3. MTG complexes with plexin and RI

In functioning as scaffolding proteins that target PKA-phosphorylation, AKAPs will often simultaneously bind the R subunits of PKA and PKA substrate(s) [23,24]. To illustrate that MTG binds RIα and plexins concurrently, forming a signaling complex, we performed pulldown assays. Results indicate that MTG is capable of and necessary for forming a complex with plexins and RIα (Fig. 3, lane 2) as RIα and PlexinA3 do not interact directly (Fig. 3, lane 1).

3.4. Phosphorylation affects MTG interactions with RI and plexins

Phosphorylation is a key event in many signaling pathways, often manifesting its effects by altering binding affinities. Recent studies have demonstrated regulation of AKAP protein interactions via PKA-phosphorylation of the AKAPs. Furthermore PlexinA, the drosophila plexin that interacts with MTG family member Nervy, may be a target for PKA-phosphorylation [3,12]. In addition, tyrosine phosphorylation of plexins is important in semaphorin signaling [26]. As such, we performed studies to test the hypothesis that PKA-phosphorylation affects plexin/MTG/RI interactions. To begin,

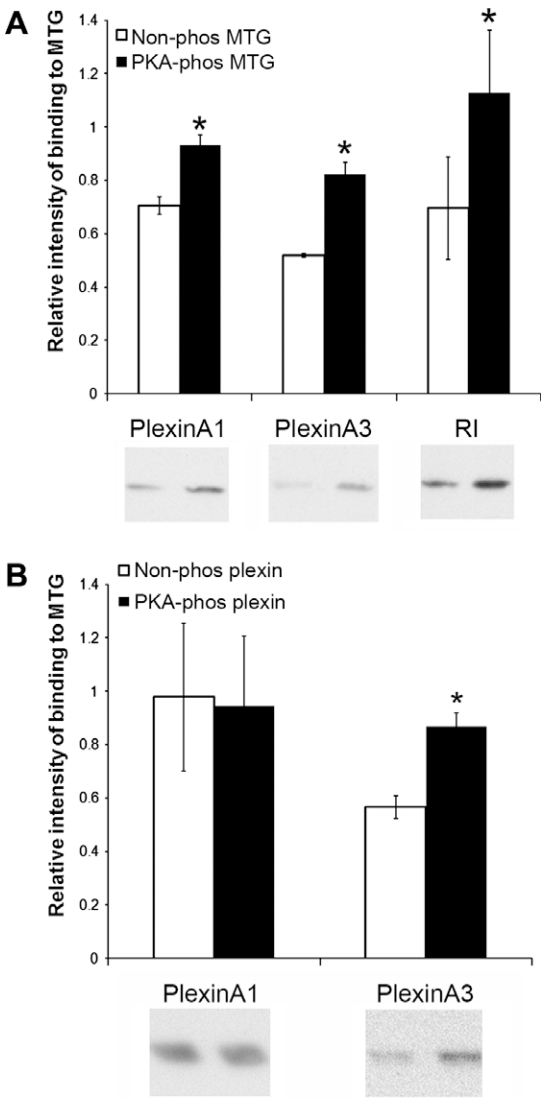

Fig. 4. PKA phosphorylation affects MTG interactions with plexins and RI. (A) MTG-S protein was immobilized on S-protein beads and either left unphosphorylated (bar 1 and lane 1 in each blot) or phosphorylated by the catalytic subunit of PKA (bar 2 and lane 2 in each blot). GST-PlexinA1 (left), GST-PlexinA3 (middle), or purified RIα (right) lysates were then incubated with the bead-bound MTG. Samples were electrophoresed, transferred and anti-GST-HRP western blots were performed to detect binding of the plexins or RIα to MTG. Phosphorylation of MTG by PKA resulted in a significant increase in binding to all proteins tested (PlexinA1 $P = 0.04$, PlexinA3 $P = 0.02$, and RIα $P = 0.02$ by t-test; $*$ denote significance). (B) PlexinA1-GST (left) or PlexinA3-GST (right) is immobilized on GST beads and either left unphosphorylated (bar 1 and lane 1 in each blot) or phosphorylated by the catalytic subunit of PKA (bar 2 and lane 2 in each blot). MTG-S protein lysate was then incubated with the bead-bound plexins. Western analysis detects binding of the MTG to plexins. Phosphorylation of PlexinA3 by PKA resulted in a significant increase in binding to MTG ($P = 0.01$ by t-test, $*$ denotes significance), while phosphorylation of PlexinA1 did not affect its interaction with MTG. The blots in A and B are each one representative of three separate experiments in which equal loading was determined by coomassie staining and western blot (anti-S protein-HRP, A, anti-GST-HRP, B). PKA-phosphorylation of proteins was confirmed using the anti-phospho-serine PKA substrate antibody. Densitometric analyses were performed using NIH Image software. Error bars are ± S.E.M.

we performed experiments to determine the sites of PKA-phosphorylation on MTG, which are S536 and S411 (see Supplementary data).

Using FL MTG, purified RIα, and the cytosolic domains of PlexinA1 or PlexinA3 (see above), we performed pulldown assays to determine whether PKA-phosphorylation of either MTG or plexins alters interactions (RIα is constitutively phosphorylated in vivo). First, MTG was immobilized on beads and PKA-phosphorylated, plexins or purified RIα were added. Binding was compared to unphosphorylated MTG. Results indicate that PKA-phosphorylation of MTG significantly increases its interaction with PlexinA1, PlexinA3, and RIα (Fig. 4A). In reverse pulldowns, plexins were immobilized and PKA phosphorylated as above, then MTG was added. Results indicate that PKA-phosphorylation of PlexinA3 increases its interaction with MTG (Fig. 4B, right), while PKA-phosphorylation of PlexinA1 has no effect on its interaction with MTG (Fig. 4B, left).

4. Conclusion

In summary, this study provides the first evidence that MTG16b is a dual AKAP capable of binding plexins. Additionally, we report that these interactions are specific (PlexinA1 and A3 are bound, while PlexinB1 is not) and can be regulated by PKA-phosphorylation. Collectively, these data suggest a possible mechanism for the targeting and integration of cAMP and semaphorin signaling in immune cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.02.007.

References

[1] Schillace, R.V. and Carr, D.W. (2006) The role of protein kinase A and A-kinase anchoring proteins in modulating T-cell activation: progress and future directions. Crit. Rev. Immunol. 26, 113–131.
[2] Torgersen, K.M., Vang, T., Abrahamsen, H., Yaqub, S. and Tasken, K. (2002) Molecular mechanisms for protein kinase A-mediated modulation of immune function. Cell Signal. 14, 1–9.
[3] Fiedler, S.E., Bajpai, M. and Carr, D.W. (2008) Identification and characterization of RHOA-interacting proteins in bovine spermatozoa. Biol. Reprod. 78, 184–192.
[4] Schillace, R.V., Andrews, S.F., Liberty, G.A., Davey, M.P. and Carr, D.W. (2002) Identification and characterization of myeloid translocation gene 16b as a novel a kinase anchoring protein in T lymphocytes. J. Immunol. 168, 1590–1599.
[5] Schillace, R.V., Miller, C.L., Pisenti, N., Grotzke, J.E., Swarbrick, G.M., Lewinsohn, D.M. and Carr, D.W. (2009) A-kinase anchoring in dendritic cells is required for antigen presentation. PLoS One 4, e4807.
[6] Rossetti, S., Hoogeveen, A.T. and Sacchi, N. (2004) The MTG proteins: chromatin repression players with a passion for networking. Genomics 84, 1–9.
[7] Fukuyama, T., Sueoka, E., Sugio, Y., Otsuka, T., Niho, Y., Akagi, K. and Kozu, T. (2001) MTG8 proto-oncoprotein interacts with the regulatory subunit of type II cyclic AMP-dependent protein kinase in lymphocytes. Oncogene 20, 6225–6232.
[8] Skalhegg, B.S., Tasken, K., Hansson, V., Huitfeldt, H.S., Jahnsen, T. and Lea, T. (1994) Location of cAMP-dependent protein kinase type I with the TCR-CD3 complex. Science 263, 84–87.
[9] Skalhegg, B.S., Landmark, B.F., Doskeland, S.O., Hansson, V., Lea, T. and Jahnsen, T. (1992) Cyclic AMP-dependent protein kinase type I mediates the inhibitory effects of 3′,5′-cyclic adenosine monophosphate on cell replication in human T lymphocytes. J. Biol. Chem. 267, 15707–15714.
[10] Song, H.J. and Poo, M.M. (1999) Signal transduction underlying growth cone guidance by diffusible factors. Curr. Opin. Neurobiol. 9, 355–363.
[11] Terman, J.R. and Kolodkin, A.L. (2004) Nervy links protein kinase a to plexin-mediated semaphorin repulsion. Science 303, 1204–1207.
[12] Bashaw, G.J. (2004) Semaphorin signaling unplugged; a nervy AKAP cAMP(s) out on plexin. Neuron 42, 363–366.
[13] Kikutani, H. and Kumanogoh, A. (2003) Semaphorins in interactions between T cells and antigen-presenting cells. Nat. Rev. Immunol. 3, 159–167.
[14] Dustin, M.L. and Colman, D.R. (2002) Neural and immunological synaptic relations. Science 298, 785–789.

[15] Takegahara, N., Kumanogoh, A. and Kikutani, H. (2005) Semaphorins: a new class of immunoregulatory molecules. Philos. Trans. Royal Soc. Lond. B: Biol. Sci. 360, 1673–1680.
[16] O'Connor, B.P. and Ting, J.P. (2008) The evolving role of semaphorins and plexins in the immune system: Plexin-A1 regulation of dendritic cell function. Immunol. Res. 41, 217–222.
[17] Moretti, S., Procopio, A., Boemi, M. and Catalano, A. (2006) Neuronal semaphorins regulate a primary immune response. Curr. Neurovasc. Res. 3, 295–305.
[18] Takegahara, N. et al. (2006) Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. Nat. Cell Biol. 8, 615–622.
[19] Tordjman, R., Lepelletier, Y., Lemarchandel, V., Cambot, M., Gaulard, P., Hermine, O. and Romeo, P.H. (2002) A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response. Nat. Immunol. 3, 477–482.
[20] Walzer, T., Galibert, L. and De Smedt, T. (2005) Dendritic cell function in mice lacking Plexin C1. Int. Immunol. 17, 943–950.
[21] Wong, A.W. et al. (2003) CIITA-regulated plexin-A1 affects T-cell-dendritic cell interactions. Nat. Immunol. 4, 891–898.
[22] Yamamoto, M. et al. (2008) Plexin-A4 negatively regulates T lymphocyte responses. Int. Immunol. 20, 413–420.
[23] Asirvatham, A.L., Galligan, S.G., Schillace, R.V., Davey, M.P., Vasta, V., Beavo, J.A. and Carr, D.W. (2004) A-kinase anchoring proteins interact with phosphodiesterases in T lymphocyte cell lines. J. Immunol. 173, 4806–4814.
[24] Bajpai, M., Fiedler, S.E., Huang, Z., Vijayaraghavan, S., Olson, G.E., Livera, G., Conti, M. and Carr, D.W. (2006) AKAP3 selectively binds PDE4A isoforms in bovine spermatozoa. Biol. Reprod. 74, 109–118.
[25] Jarnaess, E., Ruppelt, A., Stokka, A.J., Lygren, B., Scott, J.D. and Tasken, K. (2008) Dual specificity A-kinase anchoring proteins (AKAPs) contain an additional binding region that enhances targeting of protein kinase A type I. J. Biol. Chem. 283, 33708–33718.
[26] Franco, M. and Tamagnone, L. (2008) Tyrosine phosphorylation in semaphorin signalling: shifting into overdrive. EMBO Rep. 9, 865–871.